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Identification of phytanoyl-CoA ligase as a distinct acyl-CoA ligase in peroxisomes from cultured human skin fibroblasts

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Phytanic acid accumulates in excessive amounts in Refsum disease, a rare neurological disorder, due to a defect in its α-oxidation enzyme system in peroxisomes. The activation of phytanic acid to phytanoyl-CoA by phytanoyl-CoA ligase is a prerequisite for its α-oxidation. The studies described in this manuscript report that phytanoyl-CoA ligase in peroxisomes is an enzyme distinct from the previously reported acyl-CoA ligases.

Phytanoyl-CoA ligase; Peroxisome; Phytanic acid; Human skin fibroblast

1. INTRODUCTION

Phytanic acid (3,7,11,15-tetramethylhexadecanoic acid), a highly branched fatty acid, accumulates in excessive amounts in Refsum disease [1] and in diseases which lack peroxisomes [2,3]. The defect in the α-oxidation of phytanic acid is at the level of the conversion of phytanic acid to α-hydroxyphytanic acid [1,4,5]. Because of the presence of a methyl group at the β-carbon, phytanic acid does not undergo β-oxidation but is first α-oxidized to pristanic acid [4–6]. The oxidation of [1-^{14}C]phytanic acid to pristanic acid in subcellular fractions purified by Nycodenz gradient from rat (liver and cultured skin fibroblasts) and human (liver and cultured skin fibroblast) tissues demonstrated that phytanic acid is oxidized in peroxisomes in humans [7,8] and in mitochondria in rodents [6,8]. The higher specific activity of phytanic acid oxidation in peroxisomes than that in other organelles [7,8], and the deficient α-oxidation of phytanic acid, in monolayers of cultured fibroblasts [5,9,10], cellular homogenates and in isolated peroxisomes [11] from cultured skin fibroblasts from patients with Refsum disease, and in cultured skin fibroblasts from patients with diseases which lack peroxisomes (e.g. Zellweger syndrome [9]), support the conclusion that α-oxidation of phytanic acid to pristanic acid in humans is a peroxisomal function.

The activation of phytanic acid to phytanoyl-CoA is the initial and obligatory step in the oxidation of phytanic acid [7,8]. Fatty acids of different chain-length are activated to acyl-CoA derivatives by different acyl-CoA ligases [12]. For example, long-chain fatty acids (C_{16}–C_{22}) and very long-chain fatty acids (> C_{22}) are activated by palmitoyl-CoA ligase [13] and lignoceroyl-CoA ligase [14,15], respectively. At the subcellular level, palmitoyl-CoA ligase (76 kDa) is present in peroxisomes, mitochondria and endoplasmic reticulum (ER) [13] in contrast to lignoceroyl-CoA ligase which is present in peroxisomes and ER [14–18]. At present it is not known whether phytanoyl-CoA ligase activity in peroxisomes is derived from palmitoyl-CoA ligase or lignoceroyl-CoA ligase or exists as a distinct enzyme.

We examined the subcellular distribution of phytanoyl-CoA ligase in human (liver and cultured skin fibroblasts) and rat (liver and cultured skin fibroblasts) tissues. We also studied the properties of phytanoyl-CoA ligase in peroxisomes from human skin fibroblasts. These studies demonstrate that phytanoyl-CoA ligase in peroxisomes is a distinct enzyme from palmitoyl-CoA ligase and lignoceroyl-CoA ligase.

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2. MATERIALS AND METHODS

Nycodenz was obtained from Accurate Chemical and Scientific Corp., Westbury, N.Y., ATP and CoASH were purchased from P-L Biochemicals, Milwaukee, WI. [1-^{14}C]Phytanic acid (55 mCi/mmol) was purchased from Amersham International, Arlington Heights, IL, and [1-^{14}C]palmitic acid was from New England Nuclear, Boston, MA. [1-^{14}C]Lignoceric acid (58.7 mCi/mmol) was synthesized from tricosanoylibromide and K^{14}CN as described previously [20].

Peroxisomes from rat (liver and cultured skin fibroblasts) and human (liver and cultured skin fibroblasts) were isolated according to the procedures described previously [19,20]. The gradient fractions were analyzed for marker enzymes for different subcellular organelles; catalase for peroxisomes, cytochrome *c* oxidase for mitochondria and NADPH cytochrome *c* reductase for ER. Acyl-CoA ligase activities for different fatty acids was measured as described previously [21].

3. RESULTS AND DISCUSSION

We have recently demonstrated that in human tissues phytanic acid is oxidized in peroxisomes and in rat tissues in mitochondria, and that the activation of phytanic acid to phytanoyl-CoA is the initial and obligatory step in its oxidation [7,8]. Fig. 1 shows the phytanoyl-CoA ligase activities in different subcellular organelles isolated from human (liver and cultured skin fibroblasts) and rat (liver and cultured skin fibroblasts) tissues by isopycnic density gradient centrifugation. In human liver and cultured skin fibroblasts phytanoyl-CoA ligase had a trimodal distribution in peroxisomes, mitochondria and ER (Fig. 1). The specific activity of phytanoyl-CoA ligase in peroxisomes from human liver and fibroblasts was 12- and 8-times higher, respectively, than that in mitochondria (Table I). In rat tissues (liver and cultured rat skin fibroblasts) the phytanoyl-CoA ligase activity also had a trimodal distribution in ER, mitochondria and peroxisomes, however, the specific activity in mitochondria from rat liver and rat fibroblasts was 7- and 8-times higher than that observed in peroxisomes (Fig. 1 and Table I). The higher specific activity of phytanoyl-CoA ligase in peroxisomes from human tissues and mitochondria from rat tissues (Table I and Fig. 1) parallels the phytanic acid α-oxidation activities in these species [6–8]. These results suggest that the subcellular distribution of phytanoyl-CoA ligases control the phytanic acid oxidation by their ability to provide the phytanoyl-CoA for α-oxidation in these organelles. The acyl-CoA ligases in ER are considered to be responsible for providing acyl-CoA derivatives for the biogenesis of complex lipid [12,13]. The ER from rat tissues had 6- to 9-fold higher specific activity than ER from human tissues (Table I) suggesting that as compared to humans, the rat tissues should have higher amounts of phytanic acid containing lipids. Consistent with the function of acyl-CoA ligases in ER the rat tissues do in fact contain higher amounts of phytanic acid in triglycerides as compared to humans [1]. The phytanoyl-CoA ligase activity in mitochondria and ER was demonstrated previously [22]. This is the first demonstration of phytanoyl-CoA ligase activity in peroxisomes.

This differential distribution and different specific activity of phytanoyl-CoA ligase (Fig. 1 and Table I) compared to those of palmitoyl-CoA ligase and lignoceroyl-CoA ligase [14–20] in different subcellular organelles suggests that phytanoyl-CoA ligase is an enzyme distinct from the previously described palmitoyl-CoA ligase and lignoceroyl-CoA ligase. The pattern of specific activities of palmitoyl-CoA ligase and lignoceroyl-CoA ligase in peroxisomes, mitochondria and ER from cultured skin fibroblasts [18–20] and rat liver [17] are relatively similar. In contrast the specific activities of phytanoyl-CoA ligase in subcellular organelles from human tissues are different from those of rat tissues (Table I). The fact that the specific activity of phytanoyl-CoA ligase in peroxisomes from human cultured skin fibroblasts is 8-times higher than that in rat liver peroxisomes suggests that phytanoyl-CoA ligase activity in human peroxisomes is not derived from palmitoyl-CoA ligase or lignoceroyl-CoA ligase.

The conclusion that phytanoyl-CoA ligase in humans is an enzyme distinct from palmitoyl-CoA and lignoceroyl-CoA ligases was further established by the comparison of enzyme activities of acyl-CoA ligases for palmitic, lignoceric and phytanic acids when peroxisomes isolated from cultured human skin fibroblasts were treated with protease (trypsin), detergent (Triton X-100) and antibodies to palmitoyl-CoA ligase (Fig. 2). The observations that antibodies against purified palmitoyl-CoA ligase inhibit only the palmitoyl-CoA ligase activity, with no effect on the activities of phytanoyl-CoA and lignoceroyl-CoA ligases, suggests that phytanoyl-CoA ligase is a different protein from palmitoyl-CoA ligase (Fig. 2A). Moreover, the loss of activity of phytanoyl-CoA ligase, but not of palmitoyl-CoA ligase or lignoceroyl-CoA ligase, when peroxisomes were treated with detergent (Triton X-100) also support the conclusion that phytanoyl-CoA ligase is a different enzyme from palmitoyl-CoA ligase (Fig. 2B). The activity of lignoceroyl-CoA ligase was 3-times higher in detergent-treated peroxisomes (Fig. 2B). These results are consistent with our previous observations that in peroxisomes the active site of palmitoyl-CoA ligase is on the cytoplasmic surface and that of lignoceroyl-CoA ligase is on the luminal surface [23]. The disruption of the integrity of peroxisomes with Triton X-100 increased the activity of lignoceroyl-CoA ligase but not of

Table I

Specific activity of phytanoyl CoA ligase (nmol/h/mg protein) in different subcellular organelles isolated from different tissues

	PN fraction	Peroxisome	Mitochondria	Microsome
Human liver	1.46 ± 0.36	15.22 ± 1.26	1.29 ± 0.18	2.27 ± 0.11
Human fibroblast	1.34 ± 0.2	10.89 ± 1.05	1.45 ± 0.02	2.31 ± 0.31
Rat liver	6.56 ± 1.08	1.40 ± 0.18	9.63 ± 1.23	20.38 ± 2.43
Rat fibroblast	4.76 ± 0.72	1.02 ± 0.14	8.05 ± 0.96	13.41 ± 3.2

The phytanoyl-CoA ligase activity was measured as described previously [20]. The results are expressed as mean ± S.D. from three different cell lines and livers from rat and humans.

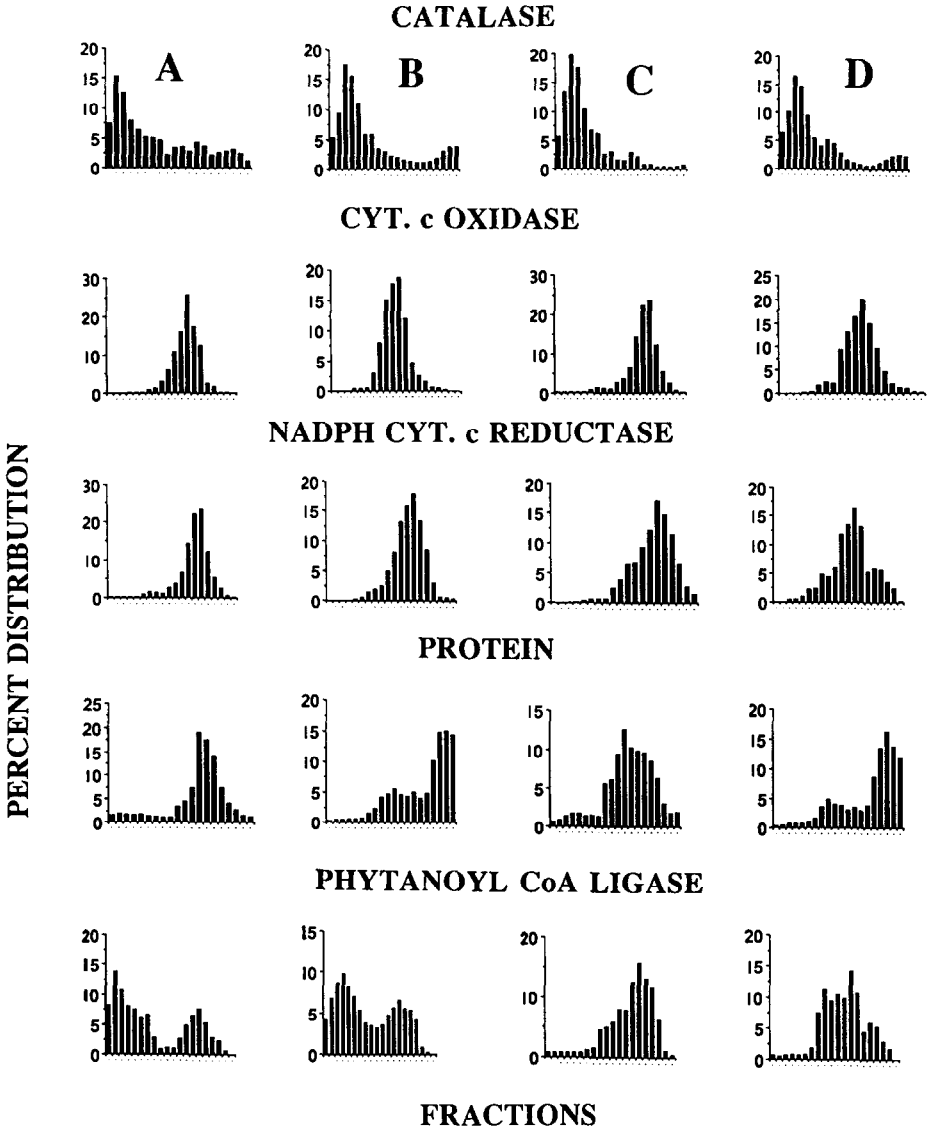

Fig. 1. Subcellular localization in phytanoyl-CoA ligase in rat and human tissues. Liver (human and rat) and cultured skin fibroblasts (human and rat) were fractionated according to the procedures described previously [21]. The distribution of subcellular organelles in the gradient were identified by their marker enzymes; catalase for peroxisomes, cytochrome *c* oxidase for mitochondria and NADPH cytochrome *c* reductase for endoplasmic reticulum. These results are the average of two gradients of each. Panels A–D represent gradient profiles of human liver, human fibroblasts, rat liver and rat fibroblasts, respectively.

palmitoyl-CoA ligase activity. The relative loss of phytanoyl-CoA ligase activity as compared to the increase in lignoceroyl-CoA ligase activity suggests that phytanoyl-CoA ligase activity in human skin fibroblasts is not derived from lignoceroyl-CoA ligase. Treatment of intact peroxisomes with trypsin inhibited the activities of both phytanoyl-CoA and palmitoyl-CoA ligases but had little effect on lignoceroyl-CoA ligase activity (Fig. 2C). The inhibition of phytanoyl-CoA ligase by Triton X-100 (Fig. 2B) and trypsin (Fig. 2C), but not of lignoceroyl-CoA ligase activity, supports the conclusion that in peroxisomes phytanoyl-CoA ligase activity is derived from an enzyme distinct from lignoceroyl-CoA ligase. This conclusion is further supported by the previous observations that lignoceroyl-CoA ligase and oxidation of lignoceric acid is deficient in peroxisomes from patients with X-linked adrenoleukodystrophy (X-ALD) [18–20]. However, the peroxisomes from X-ALD have normal activity for oxidation of phytanic acid [8,9]. Overall these studies clearly demonstrate that phytanoyl-CoA ligase in peroxisomes from human skin fibroblasts is a distinctly different enzyme from palmitoyl-CoA ligase and lignoceroyl-CoA ligase. However, at present it is not known whether phytanoyl-CoA ligase activities in peroxisomes, mitochondria and microsomes are derived from the same or different enzymes.

It is now recognized that peroxisomes perform major metabolic functions (e.g. synthesis of plasmalogens and

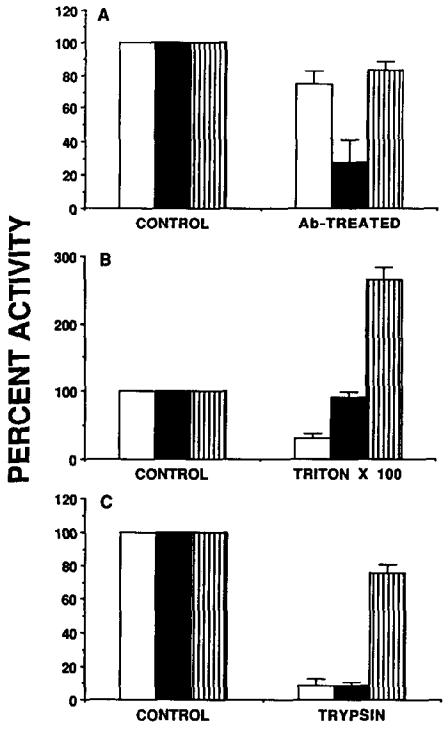

Fig. 2. (A) Effect of antisera against palmitoyl-CoA ligase on acyl-CoA ligase activities in peroxisomes. The acyl-CoA ligase activities in isolated peroxisomes from cultured skin fibroblasts for phytanic, palmitic and lignoceric were measured as described previously [20,23]. The ratio of peroxisomes-to-antibodies against palmitoyl-CoA ligase was 1:3 and controls contained the same amount of preimmune sera. This mixture was stirred for 2 h at 4°C followed by addition of 11% protein-A–Sepharose to a final concentration of 1%. The resulting mixture was stirred for 30 min and then centrifuged at 3,000 rpm for 15 min to remove sediment. Supernatant was used for ligase assays. 100% activities for phytanoyl-CoA, palmitoyl-CoA and lignoceroyl-CoA ligase were 9.05 ± 0.95, 21.85 ± 2.06 and 0.76 ± 0.07 nmol/h/mg protein, respectively. (B) Effect of Triton X-100 on Acyl-CoA ligase activities in peroxisomes. The peroxisomes from cultured human skin fibroblasts were treated with 0.05% Triton X-100 in a buffer containing 0.25 M sucrose, 1 mM EDTA-Na_2 and 3 mM imidazole, pH 7.4, for 30 min at 4°C prior to estimation of acyl-CoA ligase activities. 100% activities for phytanoyl-CoA, palmitoyl-CoA and lignoceroyl-CoA ligases were 10.89 ± 1.05, 23.84 ± 2.83 and 0.84 ± 0.07 nmol/h/mg protein, respectively. (C) Effect of trypsin on Acyl-CoA ligase activities in peroxisomes. Intact peroxisomes were treated with trypsin (trypsin:peroxisome ratio, 1:20 by protein) for 20 min at 37°C. The trypsin activity was inhibited by the addition of soybean trypsin inhibitor (trypsin:soybean trypsin inhibitor, 1:2 by protein). 100% activities for phytanoyl-CoA, palmitoyl-CoA and lignoceroyl-CoA ligases were 10.89 ± 1.05, 23.84 ± 2.83 and 0.84 ± 0.07 nmol/h/mg protein, respectively.

bile acids and oxidation of very long-chain, branched-chain, and dicarboxylic fatty acids [2,3]. Moreover, in the past 10 years 15 diseases associated with the dysfunction of peroxisomes have been identified [2,3]. This is the first demonstration of phytanoyl-CoA ligase activity in peroxisomes. The identification of the enzyme (phytanoyl-CoA ligase) for activation of phytanic acid and the possible regulation of the oxidation of phytanic acid by phytanoyl-CoA ligase underlines the importance of phytanoyl-CoA ligase in the metabolism of phytanic acid in normal and disease states with abnormality in phytanic acid catabolism (e.g. Refsum disease, Rhizomelic chondrodysplasia punctata and diseases with defects in the assembly of peroxisomes).

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